

Pollen Viability and Pollen Tube Attrition in Cranberry (*Vaccinium macrocarpon* Aiton)

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Abstract

The content of mature seed in a cranberry fruit increases with stigmatic pollen load. On average, however, only two seeds result for every tetrad of pollen deposited. What then is the fate of the two remaining pollen grains fused in each tetrad? Germination *in vitro* revealed that most of the grains in every tetrad were viable and could initiate a pollen tube (90% of tetrads generated 3-4 pollen tubes). Using a semi-vivo technique, excised styles of receptive pistils were implanted in agar, dosed with two, four or many pollen tetrads, and incubated for two days. On average, the counts of pollen tubes exiting the cut style bases averaged one quarter the counts of tetrads applied to stigmas; doubling the pollen load doubled the counts of tubes, with heaped pollen loads yielding >20 pollen tubes. Gametophytic selection via pollen tube attrition in the style appears to explain the discrepancy between stigmatic pollen load and the count of mature seeds in a cranberry fruit.

INTRODUCTION

Cranberry fruits inevitably contain seeds, the result of pollination and subsequent ovule fertilization (Eck 1986; Marucci 1967). Commercial cultivars are self-fertile (Sarracino and Vorsa, 1991), but rarely capable of passive autopolpollination (MacKenzie, 1994). Without pollinators and pollination, fruits rarely form (Cane and Schiffhauer, 2003; Marucci, 1967). Supplemental manual pollination increased field fruit set of 'Searles' cranberries, indicating that yield could be pollination limited (Birrenkott and Stang, 1989). Larger stigmatic loads of pollen yield larger, seedier cranberry fruits (Cane and Schiffhauer, 2003). Nonetheless, many pollen grains received by a receptive stigma of cranberry do not contribute to a fruit's complement of mature seeds. On average, two seeds mature for every four pollen grains received (Cane and Schiffhauer, 2003). Perhaps only two grains per tetrad are viable. Alternatively, perhaps all grains generate pollen tubes, but there is later selective abortion of developing seeds, as happens through post-fertilization self-incompatibility in taxa such as *Calluna* (Mahy and Jacquemart 1999). Birrenkott and Stang (1989) concluded that fruiting in cranberry "appears to be limited by insufficient pollen tube growth". If a greater fraction of received pollen was to fertilize ovules and produce mature seeds, then floral visits by mediocre pollinators, such as nectar-foraging honey bees, would result in more frequent fruit set (Cane and Schiffhauer 2003).

The purpose of this study was to examine male reproductive attrition in cranberry, focusing on the fertility of pollen, the distribution of fertile grains among tetrads, and pollen tube initiation and growth. To obtain replicates and visualize pollen tube elongation, I adapted a semi-vivo stylar culture technique that had been used to survey cultivar cross-compatibility in pear (Zhang and Hiratsuka 1999).

METHODS AND MATERIALS

Twelve ramets of *Vaccinium macrocarpon* Aiton representing a cross between 'Ben Lear' × 'Stevens' were grown at the University of Wisconsin, Madison USA from cuttings rooted in peat. Once flower buds had formed, plants were shipped airfreight to Logan, Utah USA in late May. They were then maintained in an unheated glasshouse until flowering.

To evaluate pollen viability, a Petri dish was prepared with autoclaved 1% agarose gel imbued with 10% sucrose and 100 ppm boric acid (Huang and Johnson, 1996; Kearns and Inouye, 1993). Fresh pollen was released from dehiscing anthers by sonication using a tuning fork, depositing pollen directly on the agar surface. The dish was sealed, inverted, and incubated in the dark at 30EC for 20h. One hundred tetrads were viewed microscopically along a transect across the dish, counting the numbers of pollen tubes emanating from each tetrad.

To visualize pollen tubes penetrating the floral pistil, a semi-vivo technique was used (Zhang and Hiratsuka, 1999). Flowers of glasshouse plants were emasculated and monitored for stigmas secreting a clear viscous fluid, indicative of receptivity. For each receptive flower, a stigma and distal 2/3 of its style was excised, held in featherweight spring steel forceps (used by entomologists), and a fixed count or mass of pollen tetrads was daubed onto the stigma from the surface of a glass slide that had been dusted with fresh pollen. The cut end of the style was then immediately implanted in sucrose agar formulated as above. Dishes were then sealed and incubated at 22EC for 45h (Rigby and Dana 1972). Pollen tubes were observed growing into the agar from the cut ends of the styles. Data were analyzed by log-likelihood contingency tests, grouping pollen tube counts in bins of 0, 25, 50, 75 and 100% of the pollen loads that were applied.

RESULTS AND DISCUSSION

Most cranberry pollen grains (86%) initiated pollen tubes visible on the agar surface. Only one of 100 tetrads counted produced no pollen tubes, whereas 59 of 100 tetrads generated 4 pollen tubes. Although the median and modal pollen tetrad produced 4 pollen tubes, significantly less than 100% pollen viability was observed ($\chi^2_{0.05 [1]} = 242$, $p < 0.001$). Comparable high pollen viability was reported for three cultivars of southern highbush blueberry (mean 82-93%), whether measured by in vitro pollen tube initiation or histochemical staining with fluorescein diacetate (Huang and Johnson, 1996). In vitro methods such as these sometimes underestimate pollen viability (Kearns and Inouye, 1993) through mutual inhibition of clumped grains. However, given that most tetrads generated 4 pollen tubes, it seems unlikely that cranberry pollen grains inhibited each other's germination, at least for the small numbers of tetrads needed to maximize fruit set ($n=6$) and size ($n=8$) (Cane and Schiffhauer, 2003). Cranberry pollen fertility is high, far exceeding the numbers of mature seeds sired for a given stigmatic pollen load.

Significantly fewer pollen tubes emanated from the cut styles than was predicted from in vitro pollen germination ($\chi^2_{0.05 [4]} = 15$, $p \leq 0.005$). On average, for styles receiving two tetrads, each tetrad produced a single pollen tube that grew at least to mid-style (24% of pollen grains, $n = 23$ styles, range 0-75%). The ratio of pollen deposited to tubes generated remained the same with a doubling of the pollen load to four tetrads (22% of pollen grains, $n = 12$ styles, range 0-69%), consistent with a linear response between stigmatic load size and resulting pollen tubes generated ($\chi^2_{0.05 [4]} = 3.1$, $p > 0.5$). For a few styles, pollen loads of two or four tetrads did generate about as many pollen tubes as predicted by pollen viability (6 and 11 tubes, respectively), showing that the full potential for pollen tube generation will be occasionally expressed. Based on these experiments, male reproductive attrition for cranberries is frequent, apparently manifesting as maternal inhibition of either pollen germination on the stigma or retarded pollen tube elongation in the style.

Stigmas given large pollen loads yielded an average of 25 ± 11 distinguishable pollen tubes emanating from the cut ends of styles. More tubes existed but could not be reliably distinguished in the tangle of emerging tubes. Only one of these 12 styles lacked pollen tubes. These values correspond well with seed production in the manual pollination trials of Cane and Schiffhauer (2003), for which maximum fruit set (80%) and seeds per fruit (24) were achieved with stigmatic loads of 16-32 pollen tetrads.

CONCLUSIONS

The semi-vivo stylar culture technique used here appears to work well with cranberries. Semi-vivo pollinations can be interpreted after 48h. The results largely agree with those from more laborious approaches using manually pollinated flowers of living plants, which require a wait of 12 weeks for fruits to mature, measure, and to dissect for seed. Semi-vivo techniques also avoid problems of inter-fruit competition for maternal resources, which has been shown to influence the likelihood of fruit set in cranberry (Birrenkott and Stang, 1989). The semi-vivo technique should also be convenient for cranberry pollination experiments that require many replicates, or microscopic application and observation of treatments, such as inhibition of pollen germination or tube elongation by known doses of fungicides deposited on the stigma (e.g. Yi et al., 2003).

Extensive manual self- and cross-pollination trials have shown that cranberry clones are largely self-fertile (Sarracino and Vorsa, 1991). However, the counts of maturing seeds (Cane and Schiffhauer, 2003) and elongating pollen tubes produced by manual pollinations of cranberry are much less than that expected from in vitro pollen viability. Such pre-fertilization inhibition resembles incomplete genetic self-incompatibility (SI) of other angiosperms (de Nettancourt, 1977). Whether cranberry's pollen tube attrition is random or genetically selective, and if so for what traits, warrants further investigation as a strategy toward improving commercial fruit set under conditions of sometimes marginal pollination service, such as that delivered by nectar-foraging honey bees (Cane and Schiffhauer, 2001).

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